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SPIROCHETE-LIKE SPIRAL BODIES IN BACTERIAL CULTURES

PLATE 1

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While attempting to obtain pure culture of smegma spirochetes, we encountered not a little difficulty which consisted in the appearance of a large number of spiral bodies resembling spirochetes. They can be detected either under the dark field microscope or in the india ink method in the special medium after 15 hours' incubation. These bodies have a striking resemblance to the involuted form of Treponema macrodentium which Noguchi[1] found in the buccal cavity. According to this investigator, they reach their maximum growth on the 2nd or 3rd day, and afterward gradually decrease in number, the process being much quicker in the presence of other bacteria producing gas and acids. Sometimes this form does not appear at all. Because of such precarious nature, its pure culture is a most difficult task. However, once started, it is comparatively easy to transplant it.

To obtain pure culture of the spiral bodies in question, we tried in vain both the filtration method and Noguchi's purification. We also tried anaerobic plate isolation in hydrogen, using plasma-ascites medium, Noguchi's ascites-fluid agar, and Shimamine's horse serum medium, incubating at 22 C. for from 3-7 days. On examination we could not find any pure colony of the spiral bodies. We could, however, detect them mixed with Bacillus subtilis. Thus, we only succeeded in obtaining the mixed cultures of these two kinds of organisms. All the possible methods we tried failed to separate them, leading us to the conclusion that there might be some symbiotic relation between them. We had, therefore, to study the morphology of the spiral bodies under consideration in the mixed culture.

Form.—From the results of the examination of several strains of the spiral bodies under the dark field microscope or with the india ink

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[1] Jour. Exper. Med., 1912, 15, p. 81

method, we can distinguish two distinct forms: (*a*) large spirochete-like bodies, as we may provisionally call them, have a rather small number of spirals, each 2-5 mikrons in length and 1-1.5 mikrons in width; (*b*) small spirochete-like bodies with short spirals (1-1.5 mikrons in length by 1-1.2 mikrons in width), at nearly a right angle with one another. In both, the forms of the spirals are regular. The number of spirals and the length varies a great deal; the former from two to several hundred spirals, the latter four to several hundred spirals. Usually, however, they have 6-15 spirals and a length of 15-30 mikrons. They are mostly straight. Sometimes they show various forms of curvatures and sometimes they assume the shape of entangled threads or bundles. Often they give an appearance of a longitudinal split, as described by Noguchi. However, transverse divisions are not lacking.

Motility.—We have never come across any motile form.

Stainability.—The spiral bodies do not have any affinity toward ordinary anilin dyes or strong staining fluids such as carbolfuchsin, anilin, gentian violet, caustic potash-methylene blue. However, since the other portions are stained with these fluids, the spiral bodies can be detected as negatives, so to speak. They refuse to take either Giemsa's fluid or its substitute — Otani's azur-eosin solution. They can, on the contrary, be beautifully stained by Löffler's flagellum staining method and by Zettnow's[2] silver impregnation.

Resistance Against Temperature.—The mixed cultures of Bacillus subtilis and the spiral bodies under consideration were mixed with the salt solution, and exposed to various degrees of temperature during various lengths of time and then inoculated in the plasma-ascites medium. They die in 1 hour at 85 C., in half an hour at 90 C., while at 60 C. the development was checked only after heating for 1 hour for 3 days in succession. B. subtilis accompanying the spiral bodies was found to have the same fate as the latter. The result may be tabulated as follows:

TABLE 1

EFFECT OF TEMPERATURE ON THE SPIRAL BODIES AND BACTERIAL CULTURES

	60 C.	65 C.	70 C.	80 C.	85 C.	90 C.
25 minutes	+	+	+	+	+	+
30 minutes	+	+	+	+	+	—
One hour	+	+	+	+	—	—

[2] Deutsch. med. Wchnschr., 1906, 32, p. 376.

The microscopic examination of the spiral bodies in the heating apparatus revealed that they considerably decrease in number after 10 minutes at 60 C. and 5 minutes at 70 C., and that they are completely disorganized after 15 minutes at 70 C. Yet when the debris was transplanted into the proper medium, a large number of the spiral bodies was seen to develop.

TABLE 2

EFFECT OF DIFFERENT TEMPERATURES ON SPIRAL BODIES

Temperature	Before Heating	5 Minutes	10 m.	15 m.	20 m.	30 m.	40 m.	50 m.	60 m.
60° C.....	+++	+++	+++	++	+	+	+	+	+
70° C.....	+++	++	+	—	—	—	—	—	—

Resistance Against Disinfectants.—The spiral bodies are disorganized immediately by 5% formalin, 5% carbolic acid and 90% alcohol, but 0.1-0.5% corrosive sublimate, 0.1% potassium permanganate, and 1% antiformin, do not disorganize them. But in all of these cases a multitude of the spiral bodies were seen to develop in the ascites-plasma medium into which thus treated material was inoculated; 5% antiformin, however, was found strong enough to kill them immediately.

TABLE 3

EFFECT OF DISINFECTANTS ON SPIRAL BODIES

Disinfectants	Before Mixing	Immediately After Mixing	5 Minutes	10 m.	15 m.	20 m.	25 m.	30 m.	Reinoculation After 30 min.
5% formalin.....	+	—	—	—	—	—	—	—	+
5% carbolic acid.....	+	—	—	—	—	—	—	—	+
90% alcohol.....	+	—	—	—	—	—	—	—	+
0.1% sublimate.....	+	+	+	+	+	+	+	+	+
0.5% sublimate.....	+	+	+	+	+	+	+	+	+
0.1% potassium permanganate	+	+	+	+	+	+	+	+	+
1% antiformin.....	+	+	+	+	+	+	+	+	+
5% antiformin.....	+	—	—	—	—	—	—	—	—

Cultivation.—We have found that the spiral bodies grow best in our plasma-ascites medium. They also grow on Noguchi's ascites agar, and Shimamine's horse serum medium. They do not develop at all in medium lacking fresh proteins.

Parenthetically we would describe the method of preparing our plasma—ascites medium: Into the horse blood serum citrate is added in proportion of 0.4% and the citrated blood is kept in the refrigerator, until the corpuscles are settled entirely. The plasma thus obtained is mixed with the same quantity of the ascites fluid (sp. gr. 1.010). A 10% solution of calcium chlorid is added to the mixture in proportion of 1 c c to 40 c c. The medium thus prepared is poured into the test tubes.

Nature of the Spiral Bodies.—From what has been described, it may be concluded that the spiral bodies correspond morphologically to spirochetes, yet they are differentiated from the latter in the following features: (*a*) lack of motion; (*b*) difference in the stainability; (*c*) powerful resistance to heat and disinfectants; (*d*) quick growth as compared with that of the genuine spirochetes, and lastly (*e*) the absolute impossibility to isolate them. Of these, the last characteristic is the most interesting and it may be worth while to describe it in more detail.

Since we failed to separate the spiral bodies from Bacillus subtilis, we reversed the process, that is, we tried to isolate B. subtilis from the spiral bodies, the culture of B. subtilis being made on the plating agar medium. The colonies were proved to be pure by microscopic examination. After their purity had been ascertained, they were inoculated in the plasma-ascites medium to see if the problematical bodies might appear. We found that they did in great number, intermingled with the subtilis bacilli. We transplanted the bacilli grown in plasma-ascites to the plain agar medium. We cultivated the subtilis for more than 20 generations on the agar. Every time the reinoculation was made, the germs were also inoculated into the plasma-ascites medium, and each time they were observed to be mixed with the spiral bodies, while on the agar medium B. subtilis alone grew.

TABLE 4

SHOWING THAT A SPECIAL RELATION EXISTS BETWEEN SPIRAL BODIES AND B. SUBTILIS

	Generation 1	Generation 2	Generation 3	Generation 4
Medium	Agar	Agar	Agar	Agar
Spiral body	—	—	—	—
Medium	Plasma	Plasma	Plasma	Plasma
Spiral body	+	+	+	+

We applied the isolation method by means of plain agar medium for 5 generations, when it was reinoculated into the plasma-ascites medium. This time again growth of the spiral bodies took place with the growth of the subtilis. From this it will be seen that not only was it impossible to isolate the spiral bodies from B. subtilis, but also that we cannot help thinking that there must be a special relation between the spiral bodies and B. subtilis.

In order to clear up the special relation, several strains of B. subtilis were inoculated into the plasma-ascites medium in which they

were seen to be mixed with the spiral bodies. We tried to grow various other species of bacteria in the plasma-ascites medium, and have found that with many species of bacteria the spiral bodies were also produced. From the results of these experiments we arrived at the conclusion that all the bacteria that are provided with flagella produce the spiral bodies in their culture. Especially numerous spiral bodies were seen to develop in the culture of B. subtilis, B. tetani, and B. anthracis. Other bacteria produced them in a very small number. Among the bacteria that are lacking flagella, B. mallei alone produced the spiral bodies, other bacteria having no flagella were found not to produce spiral bodies. From these experiments, it would seem that the spiral bodies are produced by certain species of bacteria under the certain conditions. We do not hesitate to state that the spiral bodies under consideration are not spirochetes at all.

TABLE 5

BACTERIA PROVIDED WITH FLAGELLA PRODUCE SPIRAL BODIES IN THEIR CULTURE

Species of Bacteria	Appearance of Spiral Bodies in Cultivation	Flagella
1. Staphylococcus I.	—	—
2. Staphylococcus II.	—	—
3. Pneumococcus I.	—	—
4. Pneumococcus II.	—	—
5. Streptococcus	—	—
6. Streptococcus mucosus	—	—
7. Streptococcus mucosus acidi-lacti	—	—
8. Gonococcus	—	—
9. Diphtheria bacillus	—	—
10. Pseudo-diphtheria bacillus I.	—	—
11. Pseudo-diphtheria bacillus II.	—	—
12. Bacillus bulgaricus	—	—
13. Bacillus anthracis	—	—
14. V. cholerae-gallinarum	—	—
15. B. dysenteriae	—	—
16. B. dysenteriae	—	—
17. B. dysenteriae	—	—
18. B. mallei	+	—
19. B. typhosus	+	+
20. Paratyphoid bacillus A.	+	+
21. Paratyphoid bacillus B.	+	+
22. B. coli	+	+
23. B. proteus	+	+
24. B. typhi-murium	+	+
25. B. tetani	+	+
26. B. pyocyaneus	+	+
27. V. cholerae	+	+
28. B. subtilis (smegma)	+	+
29. B. subtilis (water)	+	+

As to the transformation of various bacteria into the spiral bodies, we may say that the flagella or a portion of the bacterial bodies can undergo an unusual development under a special circumstance from the following data: (*a*) the spiral bodies are formed from flagellated

species alone; (*b*) they show the same reaction toward staining fluids as the flagella; (*c*) there exist transitional forms from the flagella to the spiral bodies as shown by Zettnow's silver impregnation method. Moreover, by applying our method to the cultivation of bacteria, the flagella may be easily detected, for B. mallei, which had been considered to have no flaggella, was found to have them.

Here it may be stated that we examined Spirocheta obermeieri, and found that it has no flagella, which is contrary to the statement of Zettnow, who affirmed the presence of flagella in that spirochete. We do not, however, insist on our view absolutely because we are not provided with the microphotographic apparatus having 6,000 magnification, with which Zettnow affirmed his view.

SUMMARY

We found spiral bodies resembling spirochetes in anaerobic cultures of various bacteria cultivated in the plasma-ascites medium, Noguchi's ascites-agar and Shimamine's horse serum medium.

The spiral bodies are nothing more than an unusual development of the flagella or parts of the bacterial bodies.

The spiral bodies seem to be identical with Noguchi's involuted forms of Treponema macrodentium.

It is suggested from our study that it is necessary to pay special attention to motility, stainability and the pure cultivation in any study of spirochetes, when associated with other bacteria. Morphology alone is not in these cases reliable.

It is further suggested that our method may be applied in the search for flagella. We have discovered the presence of flagella in B. mallei, which had been considered to have no flagellum.

EXPLANATION OF PLATE 1

Fig. 1.—Large spiral body.

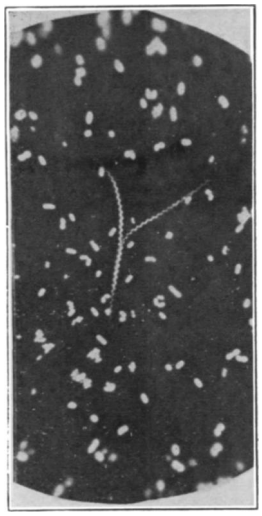
Fig. 2.—Large spiral body.

Fig. 3.—Large spiral body.

Fig. 4.—Small spiral body.

Fig. 5.—Spiral bodies and the transition forms between the spiral bodies and the flagella as seen in preparations by means of Zettnow's silver impregnation method.

Plate 1



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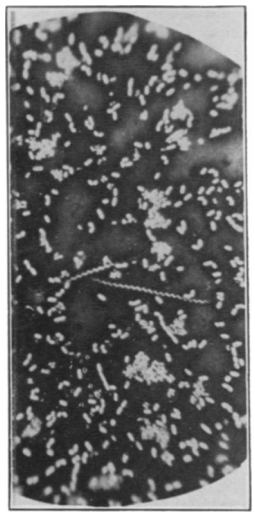

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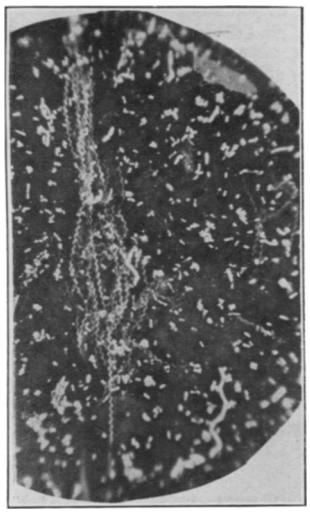

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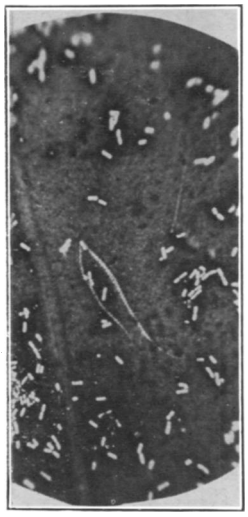

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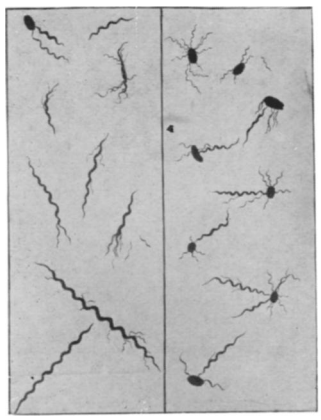

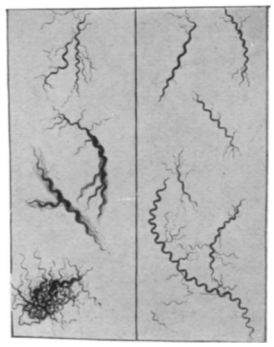

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